Using Enzymes from Recycled Fruit Wastes for Water Treatment

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ABSTRACT : 'Garbage enzyme', a mixture of fruit peels, water and brown sugar, is known as a solution with capacity of treating wastewater. This study assesses the effectiveness of different kind of garbage enzyme with different fruit peels. Pear peel garbage enzyme solution with 0g of yeast was found to be the most effective solution in reducing phosphate wastes in wastewater. Immobilized garbage enzyme solution was made in order to examine the commercial use, however there was nearly no efficacy in treating phosphate wastes in wastewater. In the anti-bacterial test, only yuja enzyme solution with 0g of yeast seemed to have anti bacterial property. However, colony count test and MIC test failed to clearly define anti bacterial property due to low consistency of the data.

Key words: Waste water treatment, garbage enzyme, phosphate wastes, ascorbic acid reduction mechanism, immobilization of garbage enzyme, anti-bacterial test

Introduction

Importance of wastewater treatment

Biological organic pollutants and excessive nutrients loading such as nitrogen and phosphorus has been a major threat to water quality (National Small Flows Clearinghouse, 1996). This is due to the increase in untreated sewage disposal and improper industrial waste management, hence introducing wastewater into water bodies. Untreated wastewater carries many harmful organisms that spread highly contagious waterborne diseases such as typhoid and cholera (National Small Flows Clearinghouse, 1996). It may also contain chemicals and heavy metals that cause significant health and environmental problems. For example, diarrheal diseases traced to contaminated water kill approximately 2 million children and cause about 900 million episodes of illness each year (National Small Flows Clearinghouse, 1996). Furthermore, high nitrate and phosphate concentrations contribute to a condition in lakes and ponds called eutrophication, the excessive growth of aquatic plants and algae (Westminster College, 2004).

The intimidating consequence emphasises again the fatal implications of untreated wastewater to humans. As such, it is of utmost importance to ensure that clean and potable water is made possible. Water treatment technology has certainly improved over the years, but it must be noted that proper sanitation, with efficient treatment, has not been practiced in many other places, especially in suburban areas in developed countries (Tan, 2006). This is due to the high cost of water treatment technology such as micro filtration and reverse osmosis. Therefore, a lower cost alternative to treat wastewater is important.

Literature Review

Wastewater from aquatic systems

Wastewater from aquatic systems such as fish farms typically consists of nitrogenous and phosphate wastes (National Small Flows Clearinghouse, 1996). In fisheries, freshwater fishes excrete primarily ammonia via the gills while excreting large amounts of diluted urine (Swann, 2006). This is because most fish eat protein-rich diets and ammonia is, metabolically, the

least expensive means of removing nitrogen produced by deamination of amino acids (Carter and Brafield, 1992). Phosphorus is also excreted by fishes after the consumption of fish diets containing phosphorus (Lazzari and Baldisserotto, 2008).

Nitrogen is a component of amino acids, nucleic acids and other cell components, while phosphorus is found primarily in nucleic acids, phospholipids and adenosine triphosphate (ATP) (Patey, Micha, Rijkenberg, Statham, Stinchcombe and Achterberg, 2008). Therefore, both nitrogen and phosphates help to promote growth of aquatic plants and algae, which can result in eutrophication (Westminster College, 2004). Therefore, it is essential to remove nitrogenous and phosphate wastes in wastewater from aquatic systems.

Garbage enzymes

Garbage Enzyme (GE) is an organic solution produced by the simple fermentation of fresh fruit or vegetable waste, brown sugar and water. The fermentation of fruit or vegetable waste is hypothetically attributed to the presence of B complex yeast and vitamin C yeast (Fazna and Meera,2013). Brown sugar is frequently used as a substrate in fermentation processes (Bae and Makoto, 2004). Fermentation also occurs in an airtight vessel to provide anaerobic conditions, where oxygen concentration is low.

The reason why the fermentation process of fruit or vegetable waste is very long, about 3 months, is because there is a very small amount of yeast found in the waste to carry out the fermentation (Choe, 2009). In this case, the enzyme concentration of the yeast is limiting. Therefore, adding more yeast will speed up the fermentation rate, shortening the fermentation process (Choe, 2009). According to Fazna and Meera (2013), fermentation of fruit or vegetable waste yielded a brownish liquid, which can be separated from the solids. The fermentation mixture can be filtered after 3 months to obtain a brownish yellow enzyme solution.

Measurement of total amount of phosphate wastes using spectrophotometry (Ascorbic acid reduction mechanism)

Spectrophotometry – Ascorbic acid reduction mechanism is typically used to measure the total quantity of phosphate wastes. Oxidative decomposition is conducted to all organic matters in sample and convert all the phosphorus compound to phosphate ions(PO_4^{3-}). Then reduction to substance resulted from phosphate ions and ammonium molybdate's reaction is conducted by ascorbic acid. Phosphomolibdenum blue is produced from this process and it makes solution color blue. Total quantity of phosphate wastes is measured through examining solution's absorbance in 880nm. This method is only available at phosphate concentration 0.04ppm to 1ppm. Following is specific procedure.

1) Mix 50㎖ of sample and 10㎖ of 4% potassium persulfate in bottle and autoclave for 30 minutes in 120℃.
2) Extract 25㎖ from 1) and mix with 2㎖ of ammonium molybdate-ascorbic acid mixture.
3) Place in 20~40℃ constant temperature water bath for about 15 minutes.
4) Check absorbance value in 880nm with UV/VIS Spectrophotometer.

※ Ammonium molybdate-ascorbic acid mixture
1) Mix ammonium molybdate 6g and antimony potassium tartrate 0.24g in 300㎖ of distilled water.
2) Put sulfuric acid(2+1) 120㎖ and ammonium sulfamate 5g with 80㎖ of distilled water.
3) Put 7.2% L-ascorbic acid solution 100㎖ and mix gently.

Beer-Lambert law

The Beer-Lambert law, also known as Beer's law relates the attenuation of light to the properties of the material through which the light is traveling. The law is commonly applied to chemical analysis measurements and used in understanding attenuation in physical optics, for photons, neutrons or rarefied gases.

$$A = \epsilon l c$$

A : The absorbance of the solution
ϵ : The molar absorptivity
l : The length of the light path
c : The concentration of the solution

From Beer-Lambert law, absorbance is proportional to concentration.

Immobilization of garbage enzymes in sodium alginate beads

Immobilization of enzymes allows to better utilize the potential of enzymes especially for commercial and industrial purposes, as immobilized enzymes offer several advantages over free enzymes. Advantages include high productivity, automation, continuous processing, precise control of the reaction, easy product recovery and the enzyme does not contaminate the final product (Swaisgood and Horton,1989).

In this project, the entrapment method using different types of gels such as sodium alginate will be used to immobilize garbage enzymes. It can be done by a very simple procedure which is of lower cost as compared to other methods such as covalent binding and membrane confinement (Mahajan, Gupta, & Sharma, 2010). This method helps to lower the cost of immobilized garbage enzyme production, making it

cost-effective and hence more suitable for commercial and industrial purposes.

Anti-bacterial test

Another way to improve the effectiveness of the filter is to have anti-bacterial properties. This will reduce the accumulation of bacterial biomasses on the surface of the filter, maintaining water quality passing through the filter. As such, the filter can be reused more frequently, lowering the cost of wastewater treatment.

The Colony Count Test will be carried out to determine the extent of bacterial growth in garbage enzyme and checks whether extract is bacteriostatic.

Objectives

1) To identify the best fruit waste, among selected types, for use to make garbage enzymes.
2) To optimize the production of garbage enzymes from the fermentation of fruit wastes selected in 1).
3) To optimize the efficiency of garbage enzymes, obtained from 2), in the treatment of wastewater.
4) To study the anti-bacterial properties of garbage enzyme.

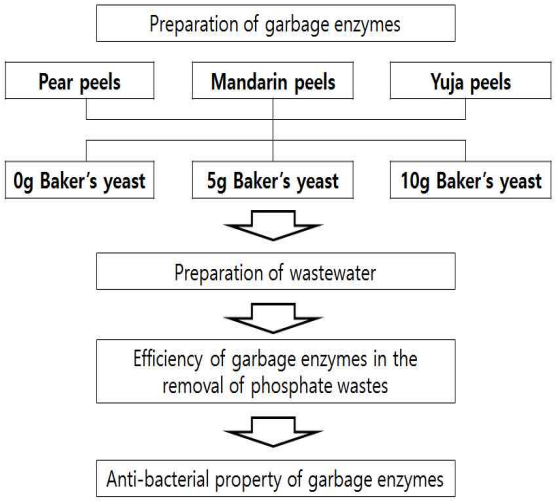
Hypotheses

1) Pear (Pyrus) peels will yield the highest garbage enzyme mixture that is most efficient in wastewater treatment compared to those obtained from mandarin (Citrus unshiu Marcov.) peels and yuja (Citrus junos) peels.
2) Garbage enzymes exhibit anti-bacterial property.

Project Outline

We used three different fruit peels (pear peals, mandarin peels and yuja peels) and each kind of fruit peel will be diversified by mass of baker's yeast added (0g, 5g, 10g). Total 9 kinds of garbage enzymes solutions were used in the experiments. As conventional methods to produce garbage enzymes is time consuming (3 months) and thus not being commercially effective, we will like to determine a method to shorten the production time of garbage enzymes. This is the first step to utilise garbage enzymes in commercial and industrial purposes.

The production of garbage enzymes, the efficiency of garbage enzymes in the removal of phosphate wastes, and the investigation of anti-bacterial properties of garbage enzymes will be progressed. Below is the visualized diagram of the project.



Experimental

1. Preparation of garbage enzymes

1.1 Fermenting garbage enzymes

Materials & Apparatus

Fruit peels	Brown sugar
Baker's yeast	Tap water
Weighing scale	Measuring cylinder
Electric blender	1.5L Plastic bottles
Buchner funnel	Duran bottles

Procedure

1) Blend 300g of pear peels to form a pulp in an electric blender.
2) To the pulp, add 100g of brown sugar, 500㎖ of tap water and 5g of baker's yeast and mix well.
3) Seal the mixture in a loosely capped plastic bottle and left to ferment. The experiment is conducted at room temperature.
4) Repeat steps 1 to 4 for mandarin peels and yuja peels respectively. Set up triplicates for each type of fruit peel.
5) After 4 weeks, brownish light yellow liquid will be seen. Vacuum filtration will be carried out using the Buchner funnel to obtain the garbage enzyme solution.

1.2 Concentrating garbage enzyme solution

Materials & Apparatus

Garbage enzyme solution	Vacuum evaporator (rotary)

Procedure

1) Place the garbage enzyme solution in the flask and then place in a rotary evaporator to concentrate the enzyme solution to 150%.

2. Preparation of wastewater

Materials & Apparatus

Ammonium chloride (NH_4Cl)	Potassium dihydrogen phosphate (KH_2PO_4)
Disodium hydrogen phosphate (Na_2HPO_4)	Urea (CH_4N_2O)
Distilled water	Measuring cylinder
Magnetic stirrer	Weighing scale
Glass rod	1L Beaker
Potassium Persulfate ($K_2S_2O_8$)	Ammonium Molybate (Ⅵ) tetrahydrate ($(NH_4)_6Mo_7O_{24}\cdot 4H_2O$)
Antimony Potassium Tartrate ($K_2Sb_2(C_4H_2O_6)_2\cdot 3H_2O$)	Sulfuric acid (H_2SO_4)
Ammonium Sulfamate ($NH_4OSO_2NH_2$)	L-Ascorbic acid ($C_6H_8O_6$)
Potassium Phosphate Monobasic (KH_2PO_4)	UV/VIS Spectrophotometer
Constant temperature water bath	Absorption cell
Autoclave	Pipette

Procedure

1) Dissolve following materials in 1L of distilled water using magnetic stirrer:
a. 225 ㎎ of Ammonium chloride
b. 150 ㎎ of Disodium hydrogen phosphate
c. 75 ㎎ of Potassium dihydrogen phosphate
d. 225 ㎎ of Urea
2) Dilute the solution in 90 folds to obtain solution where the phosphate concentration is within the acceptable reading range.
3) Analyze the concentration of phosphate ions of diluted solution using ascorbic acid reduction mechanism. The initial concentration of phosphate ions will be recorded.

3. Efficiency of garbage enzymes in the removal of phosphate wastes

3.1 Determining the level of enzyme activity

Materials & Apparatus

Wastewater	Garbage enzyme solution
Potassium Persulfate ($K_2S_2O_8$)	Ammonium Molybate (Ⅵ) tetrahydrate ($(NH_4)_6Mo_7O_{24}\cdot 4H_2O$)
Antimony Potassium Tartrate ($K_2Sb_2(C_4H_2O_6)_2\cdot 3H_2O$)	Sulfuric acid (H_2SO_4)
Ammonium Sulfamate ($NH_4OSO_2NH_2$)	L-Ascorbic acid ($C_6H_8O_6$)
Potassium Phosphate Monobasic (KH_2PO_4)	UV/VIS Spectrophotometer
Measuring cylinder	Conical flask
Weighing scale	Magnetic stirrer
Constant temperature water bath	Absorption cell
Distilled water	Autoclave
Pipette	

Procedure

1) Add 10㎖ garbage enzyme solution to 100㎖ of wastewater in a conical flask. (3 replicates and 1 control which does not contain any garbage enzyme solution)
2) Stir conical flask for 5 days.
3) After 5 days, transfer 10㎖ of each mixture into the centrifuge tubes and then centrifuge for 10 minutes at 13000 rpm.
4) Analyze the decrement of phosphate wastes comparing to initial value using ascorbic acid reduction mechanism.

3.2 Immobilization of garbage enzymes in sodium alginate beads

Materials & Apparatus

2% Sodium alginate solution	0.2M Calcium chloride solution
0.9% Sodium chloride solution	Garbage enzyme solution
Beakers	Micropipette
Sieve	Pipette tips

Procedure

1) Place 4㎖ of 2% sodium alginate solution in a 50㎖ beaker.
2) Add 4㎖ of garbage enzyme solution to the sodium alginate solution and mix gently.
3) Add the enzyme-alginate mixture dropwise into 0.2M Calcium chloride solution contained in another 50㎖ beaker.
4) Repeat step 3) until 30 beads are produced. Gently agitate the solution containing the beads.
5) Discard any beads which are significantly different in size, distorted in shape, or which float from the beaker.
6) Remove the beads from the bead mixture using sieve. Then wash beads with 0.9% of sodium chloride.
7) Place all the beads to a beaker containing 0.9% of sodium chloride to prevent the beads from drying out.

3.3 Batch Studies

Materials & Apparatus

Wastewater	Immobilized garbage enzyme
UV/VIS Spectrophotometer	Conical flask
Beakers	Micropipette
Sieve	Absorption cell

Pipette tips	Autoclave
Potassium Persulfate ($K_2S_2O_8$)	Ammonium Molybate (Ⅵ) tetrahydrate ($(NH_4)_6Mo_7O_{24}\cdot 4H_2O$)
Antimony Potassium Tartrate ($K_2Sb_2(C_4H_2O_6)_2\cdot 3H_2O$)	Sulfuric acid (H_2SO_4)
Ammonium Sulfamate ($NH_4OSO_2NH_2$)	L-Ascorbic acid ($C_6H_8O_6$)
Potassium Phosphate Monobasic (KH_2PO_4)	Constant temperature water bath

Procedure

1) Add 10 sodium alginate beads to 100㎖ of wastewater in a conical flask. (3 replicates and 1 control which does not contain any garbage enzyme solution)
2) Stir conical flask for 5 days.
3) After 5 days, transfer 10㎖ of each mixture into the centrifuge tubes and then centrifuge for 10 minutes at 13000 rpm.
4) Analyze the decrement of phosphate wastes comparing to initial value using ascorbic acid reduction mechanism.

4. Anti-bacterial test

4.1 Colony count test

Materials & Apparatus

E.coli culture	Saline solution
Garbage enzyme solution	Laminar flow hood
Bunsen burner	75% ethanol
Micropipette	LB agar plates
Shaking incubator	LB broth
Absorption cell	UV/VIS Spectrophotometer
10㎖ Tube	Incubator
Pipette tips	Parafilm

Procedure

1) Control the absorbance of E.coli culture in LB broth to 1.0 using UV/VIS Spectrophotometer at 600nm.
2) In 10㎖ tube, add 2㎖ of garbage enzyme solution to 3㎖ of E.coli culture in LB broth. (3 replicates and 1 control which contain saline solution instead of garbage enzyme)
3) Incubate the tubes overnight in the shaking incubator with shaking in 40℃.
4) Examine absorbance of each tubes using UV/VIS Spectrophotometer set at 600nm and analyze the change compared to initial absorbance.
5) Conduct serial dilution of each solution to 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} fold of initial concentration.
6) Extract 200㎕ of each diluted bacterial solution and spread onto the LB agar plates. Then incubate them overnight in the incubator.
7) Retrieve the LB agar plates next day and count the number of colonies on each plate.

Results and Discussion

1. Preparation of garbage enzymes

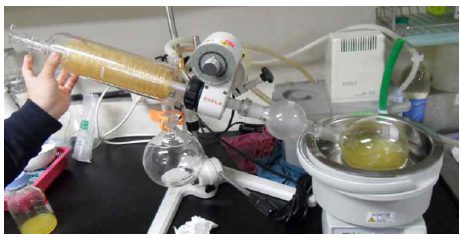
1.1 Fermenting garbage enzymes

A large amount of garbage enzyme solutions had been produced for study. After adding brown sugar, distilled water and fruit peels, we fermented the solution for about 4 weeks (2015.12.14. ~ 2016.1.15.). After fermentation, vacuum filtration was conducted and most of big sediments were removed.


Garbage enzyme


Vacuum filtration

1.2 Concentrating garbage enzyme solution


Concentration using vacuum evaporator

The garbage enzyme solution was concentrated to 150% of its initial concentration using vacuum evaporator.

2. Preparation of wastewater



Potassium dihydrogen phosphate



Disodium hydrogen phosphate

※ Concentration of phosphate wastes
Na_2HPO_4 150 ㎎ : [P] = 32.74647887323944 ppm
KH_2PO_4 75 ㎎ : [P] = 17.09558823529412 ppm
90 folds dilution : [P] = (32.74647887323944 + 17.09558823529412) ÷ 90 = 0.5538007456503729 ppm

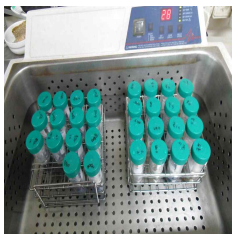
Total concentration : 0.5538007456503729 ppm

3. Efficiency of garbage enzymes in the removal of phosphate wastes

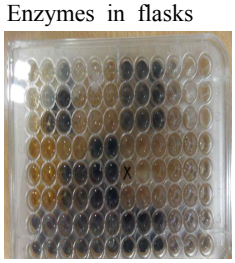
3.1 Determining the level of enzyme activity



Wastewater + Garbage Enzymes in flasks



Constant temperature water bath



Samples in absorption cell

Table 1 : Wastewater's initial absorbance value

	Distilled Water	Wastewater
1st	0.068	0.219
2nd	0.058	0.264
3rd	0.06	0.224
Average	0.062	0.23566666667
STDEV	0.0052915026221	0.02466441431

We could figure out absorbance level of phosphate wastes by subtracting distilled water's absorbance from wastewater's absorbance.
<Absorbance of 0.553800745650379 ppm of phosphate wastes in wastewater>
→ 0.23566666667 – 0.062 = 0.17366666667

※ Defining term

1st, 2nd, 3rd : Absorbance value of garbage enzyme solution with wastewater.

Initial value : Initial absorbance value of garbage enzyme solution before mixing with wastewater.

Increment : Increment of absorbance value contrasting average with initial value. *(Average − Initial value)*

Case 1-1

Pear

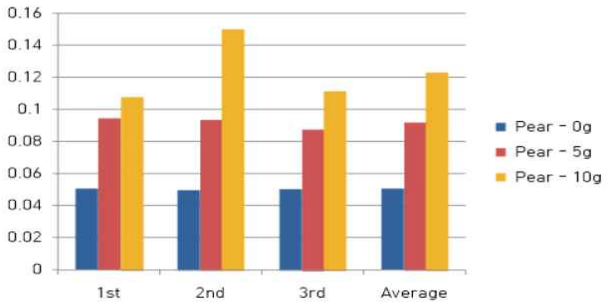
Table 2-1 : Absorbance of pear peels with wastewater

	0g - ①	0g - ②	0g - ③	5g - ①	5g - ②	5g - ③	10g - ①	10g - ②	10g - ③
1st	0.18	0.183	0.183	0.242	0.238	0.23	0.304	0.336	0.314
2nd	0.191	0.185	0.183	0.238	0.234	0.232	0.31	0.367	0.313
3rd	0.186	0.188	0.192	0.239	0.246	0.239	0.312	0.35	0.31
Average	0.185667	0.185333	0.186	0.239667	0.239333	0.233667	0.308667	0.351	0.312333
STDEV	0.005508	0.002517	0.005196	0.002082	0.00611	0.004726	0.004163	0.015524	0.002082
Inital value	0.135	0.135	0.135	0.145	0.145	0.145	0.2	0.2	0.2
Increment	0.050667	0.050333	0.051	0.094667	0.094333	0.088667	0.108667	0.151	0.112333

Table 2-2 : Absorbance increment of pear peels with wastewater

	Pear - 0g	Pear - 5g	Pear - 10g
1st	0.050666666667	0.09466666667	0.10866666667
2nd	0.050333333333	0.09433333333	0.151
3rd	0.051	0.08866666667	0.11233333333
Average	0.050666666667	0.09255555556	0.124

Figure 1 : Absorbance increment of pear peels with wastewater



Case 1-2

Yuja

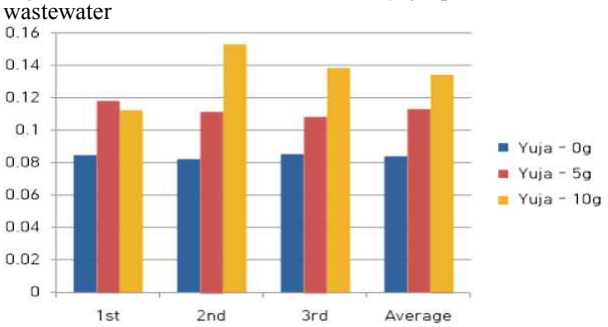
Table 3-1 : Absorbance of yuja peels with wastewater

	0g - ①	0g - ②	0g - ③	5g - ①	5g - ②	5g - ③	10g - ①	10g - ②	10g - ③
1st	0.533	0.542	0.543	0.72	0.692	0.685	1.001	1.008	1.012
2nd	0.558	0.549	0.549	0.697	0.698	0.705	0.962	1.026	0.977
3rd	0.547	0.539	0.548	0.691	0.699	0.689	0.94	0.991	0.992
Average	0.546	0.543333	0.546667	0.702667	0.696333	0.693	0.967667	1.008333	0.993667
STDEV	0.01253	0.005132	0.003215	0.015308	0.003786	0.010583	0.030892	0.017502	0.017559
Inital value	0.461	0.461	0.461	0.584	0.584	0.584	0.855	0.855	0.855
Increment	0.085	0.082333	0.085667	0.118667	0.112333	0.109	0.112667	0.153333	0.138667

Table 3-2 : Absorbance increment of yuja peels with wastewater

	Yuja - 0g	Yuja - 5g	Yuja - 10g
1st	0.085	0.11866666667	0.11266666667
2nd	0.082333333333	0.11233333333	0.15333333333
3rd	0.085666666667	0.109	0.13866666667
Average	0.084333333333	0.11333333333	0.13488888889

Figure 2 : Absorbance increment of yuja peels with wastewater



Case 1-3

Mandarin

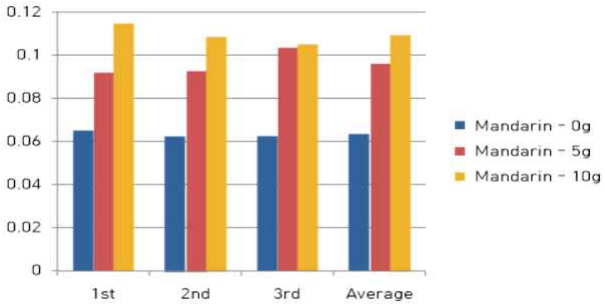
Table 4-1 : Absorbance of mandarin peels with wastewater

	0g - ①	0g - ②	0g - ③	5g - ①	5g - ②	5g - ③	10g - ①	10g - ②	10g - ③
1st	0.146	0.144	0.145	0.682	0.682	0.698	0.95	0.938	0.934
2nd	0.149	0.144	0.146	0.682	0.687	0.691	0.909	0.935	0.928
3rd	0.147	0.147	0.143	0.682	0.681	0.692	0.961	0.929	0.929
Average	0.147333	0.145	0.144667	0.682	0.683333	0.693667	0.94	0.934	0.930333
STDEV	0.001528	0.001732	0.001528	0	0.003215	0.003786	0.027404	0.004583	0.003215
Inital value	0.082	0.082	0.082	0.59	0.59	0.59	0.825	0.825	0.825
Increment	0.065333	0.063	0.062667	0.092	0.093333	0.103667	0.115	0.109	0.105333

Table 4-2 : Absorbance increment of mandarin peels with wastewater

	Mandarin - 0g	Mandarin - 5g	Mandarin - 10g
1st	0.065333333333	0.092	0.115
2nd	0.063	0.09333333333	0.109
3rd	0.062666666667	0.10366666667	0.10533333333
Average	0.063666666667	0.09633333333	0.10977777778

Figure 3 : Absorbance increment of mandarin peels with wastewater



In all three cases of garbage enzyme solution, the highest absorbance increment was detected in the case of solution with yeast 10g, following solution with yeast 5g and 0g sequently. In each of the case, average value of three trials showed consistent tendency; as amount of yeast in solution increase, increment of absorbance gets higher.

Saline Solution(Control)

Table 5 : Absorbance of saline solution with wastewater

	Control ①	Control ②	Control ③
1st	0.24	0.243	0.243
2nd	0.241	0.239	0.244
3rd	0.239	0.244	0.237
Average	0.24	0.242	0.241333
STDEV	0.001	0.002646	0.003786
Inital value	0.073	0.073	0.073
Increment	0.167	0.169	0.168333

From the Beer-Lambert law ($A = \epsilon lc$) absorbance value is proportional to concentration. From following proportional expression using increment value of each sample(y), it's possible to figure out the concentration of phosphate wastes in garbage enzyme with wastewater(x).

$$0.553800745650379 : 0.17366666667 = x : y$$

Table 6 : Concentration of phosphate and decrement of phosphate of each solution

	Phosphate (ppm)	Decrement of phosphate (ppm)
Pear - 0g	0.16156950737	0.39223123828
Pear - 5g	0.29514780622	0.25865293943
Pear - 10g	0.39542011014	0.15838063551
Yuja - 0g	0.26892819319	0.28487255247
Yuja - 5g	0.36140547701	0.19239526864
Yuja - 10g	0.43014338146	0.12365736419
Mandarin - 0g	0.2030248415	0.35077590415
Mandarin - 5g	0.30719465546	0.24660609019
Mandarin - 10g	0.35006726596	0.20373347969
Saline solution	0.5360847909	0.017715954755

Figure 4 : Decrement of phosphate waste of each solution

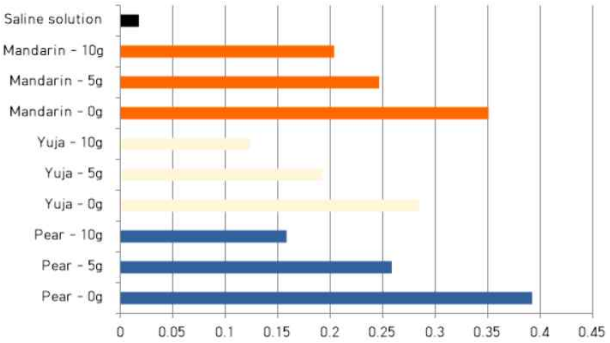


Table 6 and Figure 4 show the decrement of phosphate waste when wastewater is treated with each solution. Pear peel garbage enzyme solution with 0g of yeast reduced phosphate the most for about 0.3922 ppm. While yuja peel solution with 10g of yeast reduced phosphate the least among garbage enzyme solution for about 0.1237 ppm. Saline solution reduced phosphate the least for about 0.0177 ppm. From this result, we could conclude that pear peel garbage enzyme solution with 0g of yeast is most effective in reducing phosphate wastes in wastewater. Among garbage enzyme solution, yuja peel solution with 10g of yeast is least effective in reducing phosphate wastes in wastewater. All of the garbage enzyme solutions have capacity in getting rid of phosphate wastes contrasting to control group which is saline solution.

3.2 Immobilization of garbage enzymes in sodium alginate beads



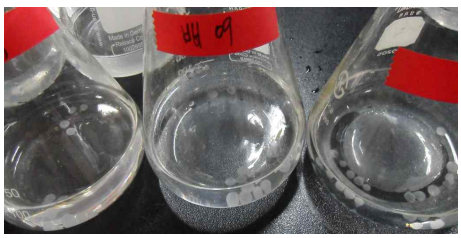
Yuja beads



Mandarin beads

All of the garbage enzyme solution was able to produce sodium alginate beads except the case of pear peel enzyme solution with 5g and 10g of yeast. Even though the size was different, all the other solution except those formed beads. However, in the case of pear peel solution with 5g and 10g of yeast, it formed spreaded shape with big distortion of its shape instead of forming bead shape. As a result, we decided to conduct batch studies excluding pear beads of 5g and 10g of yeast.

3.3 Batch Studies



Beads added in 100㎖ wastewater

Case 2-1

Pear

Table 7 : Absorbance of pear beads with wastewater

	0g - ①	0g - ②	0g - ③
1st	0.24	0.233	0.234
2nd	0.233	0.236	0.241
3rd	0.236	0.243	0.24
Average	0.2363333	0.2373333	0.2383333
STDEV	0.0035119	0.0051316	0.0037859
Inital value	0.067	0.067	0.067
Increment	0.1693333	0.1703333	0.1713333

Case 2-2

Yuja

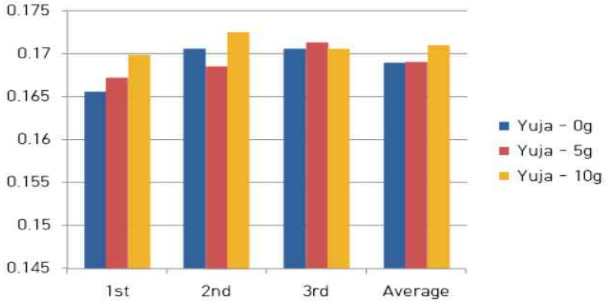
Table 8-1 : Absorbance of yuja beads with wastewater

	0g - ①	0g - ②	0g - ③	5g - ①	5g - ②	5g - ③	10g - ①	10g - ②	10g - ③
1st	0.233	0.239	0.236	0.238	0.243	0.243	0.237	0.24	0.244
2nd	0.239	0.244	0.243	0.233	0.238	0.242	0.24	0.237	0.233
3rd	0.238	0.242	0.246	0.241	0.235	0.239	0.237	0.245	0.239
Average	0.2366667	0.2416667	0.2416667	0.2373333	0.2386667	0.2413333	0.238	0.2406667	0.2386667
STDEV	0.0032146	0.0025166	0.0051316	0.0040415	0.0040415	0.0020817	0.0017321	0.0040415	0.0055076
Inital value	0.071	0.071	0.071	0.07	0.07	0.07	0.068	0.068	0.068
Increment	0.1656667	0.1706667	0.1706667	0.1673333	0.1686667	0.1713333	0.17	0.1726667	0.1706667

Table 8-2 : Absorbance increment of yuja beads with wastewater

	Yuja - 0g	Yuja - 5g	Yuja - 10g
1st	0.16566666667	0.16733333333	0.17
2nd	0.17066666667	0.16866666667	0.17266666667
3rd	0.17066666667	0.17133333333	0.17066666667
Average	0.169	0.16911111111	0.17111111111

Figure 5 : Absorbance increment of yuja beads with wastewater



Case 2-3

Mandarin

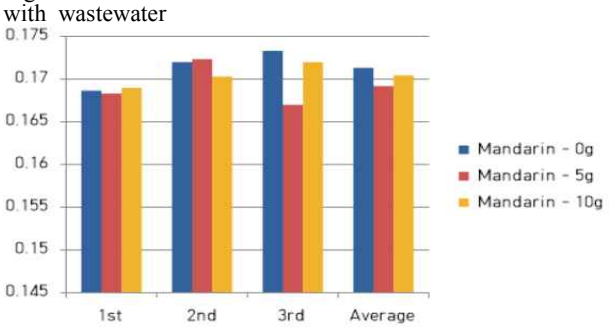
Table 9-1 : Absorbance of mandarin beads with wastewater

	0g - ①	0g - ②	0g - ③	5g - ①	5g - ②	5g - ③	10g - ①	10g - ②	10g - ③
1st	0.238	0.249	0.246	0.236	0.238	0.236	0.238	0.235	0.243
2nd	0.235	0.236	0.242	0.239	0.243	0.238	0.238	0.236	0.241
3rd	0.237	0.235	0.236	0.237	0.243	0.234	0.235	0.244	0.236
Average	0.2366667	0.24	0.2413333	0.2373333	0.2413333	0.236	0.237	0.2383333	0.24
STDEV	0.0015275	0.0078102	0.0050332	0.0015275	0.0028868	0.002	0.0017321	0.0049329	0.0036056
Inital value	0.068	0.068	0.068	0.069	0.069	0.069	0.068	0.068	0.068
Increment	0.1686667	0.172	0.1733333	0.1683333	0.1723333	0.167	0.169	0.1703333	0.172

Table 9-2 : Absorbance increment of yuja beads with wastewater

	Mandarin - 0g	Mandarin - 5g	Mandarin - 10g
1st	0.16866666667	0.16833333333	0.169
2nd	0.172	0.17233333333	0.17033333333
3rd	0.17333333333	0.167	0.172
Average	0.17133333333	0.16922222222	0.17044444444

Figure 6 : Absorbance increment of mandarin beads with wastewater



In all three cases of immobilized garbage enzyme, there were no significant difference of increment of absorbance level between samples. No special tendency was detected in relationship between amount of yeast and absorbance level or type of fruit peels and absorbance level.

Table 10 : Concentration of phosphate and decrement of phosphate of each solution

	Phosphate (ppm)	Decrement of phosphate (ppm)
Pear - 0g	0.5431711728	0.010629572853
Yuja - 0g	0.53891934366	0.014881401994
Yuja - 5g	0.53927366275	0.014527082899
Yuja - 10g	0.54565140646	0.0081493391874
Mandarin - 0g	0.54636004465	0.0074407009972
Mandarin - 5g	0.53962798185	0.014172763804
Mandarin - 10g	0.54352549189	0.010275253758
Saline solution	0.5360847909	0.017715954755

Figure 7 : Decrement of phosphate waste of each solution

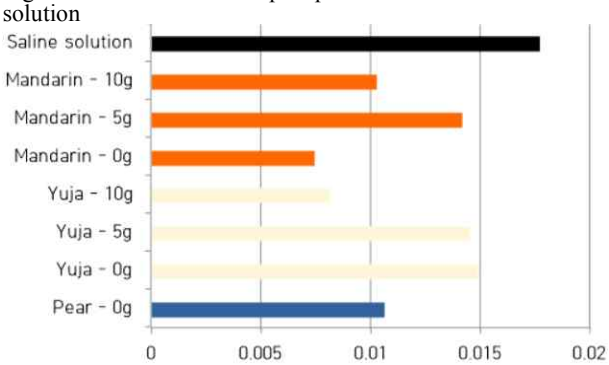


Table 10 and Figure 7 show the decrement of phosphate waste when wastewater is treated with each immobilized garbage enzyme solution. Saline solution showed the highest decrement while mandarin peel bead with 0g of yeast showed the lowest decrement of phosphate. However, there are no significant difference of decrement of phosphate waste between all the samples. Moreover, all the immobilized enzyme showed lower decrement than saline solution. From this result, we could conclude that all of the immobilized garbage enzyme solutions have nearly no efficacy in treating phosphate wastes in wastewater.

4. Anti-bacterial test

4.1 Colony count test



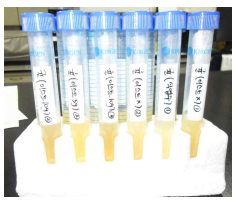
LB broth with spreaded bacterial solution



Counting colonies



Incubated *E.coli*



E.coli culture + garbage enzyme in 10㎖ tube

Case 3-1

Pear

Table 11-1 : Absorbance of *E.coli* culture with pear enzyme solution.

	Saline - ①	Saline - ②	Saline - ③	0g - ①	0g - ②	0g - ③
1st	0.896	0.996	0.921	1.08	1.152	0.982
2nd	0.911	0.938	0.936	0.937	1.091	0.979
3rd	0.957	0.953	0.978	0.982	1.102	0.992
Average	0.921333	0.962333	0.945	0.999667	1.115	0.984333
	5g - ①	5g - ②	5g - ③	10g - ①	10g - ②	10g - ③
1st	1.223	1.411	1.372	1.41	1.435	1.421
2nd	1.423	1.397	1.325	1.384	1.447	1.432
3rd	1.359	1.298	1.322	1.41	1.423	1.42
Average	1.335	1.368667	1.339667	1.401333	1.435	1.424333

Table 11-2 : The number of colony of *E.coli* culture with pear enzyme solution.

	Saline - ①	Saline - ②	Saline - ③	0g - ①	0g - ②	0g - ③
10^{-4}	x	x	28	49	108	x
10^{-6}	65	x	110	25	34	58
10^{-8}	73	31	65	x	78	x
10^{-10}	9	107	122	27	27	x
10^{-12}	x	14	x	52	x	x
	5g - ①	5g - ②	5g - ③	10g - ①	10g - ②	10g - ③
10^{-4}	83	81	x	x	x	x
10^{-6}	67	56	93	5	74	x
10^{-8}	47	14	68	50	36	26
10^{-10}	46	67	54	73	36	1
10^{-12}	45	33	32	67	32	x

Table 11-1 shows the absorbance of *E.coli* culture with pear enzyme solution. Contrasting to samples with saline solution added which is control group, absorbance increases as the amount of yeast in enzyme solution increases. This shows that none of pear enzyme solution has anti-bacterial property. In the colony count test, it is difficult to analyze the test result because there is no consistency in data.

Case 3-2

Yuja

Table 12-1 : Absorbance of *E.coli* culture with yuja enzyme solution.

	Saline - ①	Saline - ②	Saline - ③	0g - ①	0g - ②	0g - ③
1st	0.832	0.818	0.871	0.66	0.61	0.61
2nd	0.759	0.872	0.906	0.666	0.653	0.615
3rd	0.913	0.865	0.873	0.664	0.618	0.64
Average	0.834667	0.851667	0.883333	0.663333	0.627	0.621667
	5g - ①	5g - ②	5g - ③	10g - ①	10g - ②	10g - ③
1st	1.111	1.066	1.129	1.08	0.825	1.069
2nd	1.144	1.118	1.097	1.122	1.087	1.128
3rd	1.139	1.116	1.122	1.141	1.154	1.125
Average	1.131333	1.1	1.116	1.114333	1.022	1.107333

Table 12-2 : The number of colony of *E.coli* culture with yuja enzyme solution.

	Saline - ①	Saline - ②	Saline - ③	0g - ①	0g - ②	0g - ③
10^{-4}	x	x	x	x	x	x
10^{-6}	64	178	108	103	112	165
10^{-8}	0	1	4	4	0	2
10^{-10}	0	0	0	0	0	21
10^{-12}	0	0	0	0	0	1
	5g - ①	5g - ②	5g - ③	10g - ①	10g - ②	10g - ③
10^{-4}	9	7	0	0	43	0
10^{-6}	1	0	0	0	0	0
10^{-8}	0	0	0	0	0	0
10^{-10}	0	0	0	0	0	0
10^{-12}	0	0	1	0	0	0

Table 12-1 shows the absorbance of *E.coli* culture with yuja enzyme solution. Contrasting to samples with saline solution added which is control group, only absorbance of enzyme solution with no yeast was lower than control group. This shows that yuja enzyme solution with no yeast has anti-bacterial property while other two solutions do not. In the colony count test, it is difficult to analyze the test result because there is no consistency in data.

Case 3-3

Mandarin

Table 13-1 : Absorbance of *E.coli* culture with mandarin enzyme solution.

	Saline - ①	Saline - ②	Saline - ③	0g - ①	0g - ②	0g - ③
1st	1.076	0.987	0.912	1.624	1.623	1.634
2nd	0.983	0.924	1.015	1.608	1.627	1.612
3rd	0.991	0.909	1.01	1.608	1.598	1.615
Average	1.016667	0.94	0.979	1.613333	1.616	1.620333
	5g - ①	5g - ②	5g - ③	10g - ①	10g - ②	10g - ③
1st	1.34	1.359	1.361	1.318	1.332	1.329
2nd	1.322	1.337	1.364	1.31	1.293	1.315
3rd	1.342	1.343	1.374	1.337	1.31	1.087
Average	1.334667	1.346333	1.366333	1.321667	1.311667	1.243667

Table 13-2 : The number of colony of *E.coli* culture with mandarin enzyme solution.

	Saline - ①	Saline - ②	Saline - ③	0g - ①	0g - ②	0g - ③
10^{-4}	x	x	x	96	133	119
10^{-6}	x	x	84	x	x	x
10^{-8}	172	x	56	x	175	x
10^{-10}	153	x	x	233	x	241
10^{-12}	80	x	x	x	x	219
	5g - ①	5g - ②	5g - ③	10g - ①	10g - ②	10g - ③
10^{-4}	165	x	x	x	x	x
10^{-6}	x	x	x	127	x	x
10^{-8}	x	x	x	x	x	67
10^{-10}	x	x	x	x	97	132
10^{-12}	x	x	173	x	x	86

Table 13-1 shows the absorbance of *E.coli* culture with mandarin enzyme solution. Contrasting to samples with saline solution added which is control group, all of the absorbance of enzyme solution were higher than control group. This shows that none of mandarin enzyme solution has anti-bacterial property. In the colony count test, it is difficult to analyze the test result because there is no consistency in data.

Conclusion

The phase of testing the efficiency of garbage enzymes in the removal of phosphate wastes, the results indicate that pear peel garbage enzyme solution with 0g of yeast is most effective in reducing phosphate wastes in wastewater. Among garbage enzyme solution, yuja peel solution with 10g of yeast is least effective in reducing phosphate wastes in wastewater. All of the garbage enzyme solutions have capacity in getting rid of phosphate wastes contrasting to control group which is saline solution.

In the phase immobilizing garbage enzymes in sodium alginate beads, pear garbage enzyme beads was not formed.

In the phase of batch studies, all of the immobilized garbage enzyme solutions have nearly no efficacy in treating phosphate wastes in wastewater.

In the phase of testing anti bacterial property, the results indicate that no enzyme solution has anti bacterial property except yuja enzyme solution with 0g of yeast. In the case of colony count test, it was not able to analyze data because there was no consistency. MIC test also failed to find clear connection between concentration of garbage enzyme and anti bacterial property.

Following further studies should be conducted in order to determine clear effect of garbage enzyme solution. 1) Testing purification capacity of garbage enzyme for other wastes in aqua system, 2) Investigating the cause of failure in making beads with some of pear garbage enzyme solution, 3) Batch studies with more beads on it. Following is the way to supplement this study; 1) Testing the capacity in removing nitrogen wastes, 2) More accurate test on anti-bacterial property.

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